9—A CONTRIBUTION TO THE LIFE HISTORY OF MACROZAMIA REIDLEI.*

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INTRODUCTION.

No full account has been published of the reproduction of Macrozamia. Chamberlain (3) described late stages of ovule and embryo of *M. Moorei*. Light (9) 1924 gave an account of the sporophyll and young ovule with details of cell formation in the prothallus.

Miss E. R. L. Reed made collections of ovules from Kalamunda in 1928-9. Miss A. Fabre in 1930 studied some phases of the development and was the first to see the living sperms in this species, but her results were not published. I am indebted to Miss Fabre for the use of her slides, particularly those of archegonia, egg, and 2-nucleate proembryo.

The present investigation has extended over several years and except for early stages in the development of the cone, all phases of the sexual reproduction have been studied. As, however, most of the genera of the Cycads have been fully investigated and the life history found to be fairly uniform within the group, only those stages which have received least attention from other writers, or in which interesting generic differences are known to occur, are described here in detail.

*M. Reidlei (Gaud.) C. A. Gardn. = M. Fraseri. (Miq.).

The material used has been collected from Crawley and Wembley (on the coastal sand plain within a few miles of Perth), and parts of the Darling Range.

MALE AND FEMALE CONES.

Fig. 1 shows a mature female cone composed of massive sporophylls, each with the single spine characteristic of the genus. This was a large cone measuring 50 cms. by 25 cms. with a weight of 30 lbs. Cones vary greatly in size and may be only half the dimensions given.

A well developed male cone (fig. 2) measures 30 cms. or more by 15 cms. just before elongation of the axis. Mature sporophylls are shown in fig. 3. Those in the lower row were taken from the tip, centre and base respectively of the same cone and show progressive shortening of the spine down the cone. Sporangia are in groups of 3-7, 5 being the commonest number.

Female cones occur singly or 2-3 per plant, and in rare instances up to 7 cones. Male plants bear 2-5 cones as a rule but 6 or 7 are not uncommon and I have found 11 cones on one plant. Both the number of cones per plant and the size of the individual cones vary considerably from one locality to another. Plants growing in the stony soil of the Darling Range, for instance, have smaller cones than those on the coastal sand plain and produce on the average fewer cones per plant.

Development of cones.

The young cones rarely become exposed before May, although coning plants can be recognised considerably earlier by the spreading apart of the leaves and the presence of a central group of densely woolly scale leaves which enclose the young cones.

Very early stages in the development have not been examined. Even in March, when the cones are still very deeply buried, the sporophylls are well developed and ovules are about 2 mms. in diameter. In both male and female cones the sporophylls are hairy outside in the early stages, becoming glabrous later.

In a young female cone, up to about the size of that in fig. 4 which measured 15 cm., the sporophylls are long and tapering, with no marked enlargement at the base where the 2 small ovules are attached. Growth from May onwards is almost entirely restricted to the lower part, which becomes steadily broader and thicker until at maturity the sporophylls measure 6-7 cm. across by 5 cm. deep with the upper part persisting as the upturned spine.

In the male cone the numerous sporophylls are very tightly packed throughout May, June and July, each sporophyll being marked by the impression of the sporangia from the one above. Shortly before the pollen is ripe the sporophyll increases in thickness at the peripheral end, and its rate of growth, particularly in a radial direction, exceeds that of the sporangia, which in consequence become less crowded and round up into a more regular shape as the pressure on the sides and top is relieved.

About the same time as the first sporangia open, there is a rapid elongation of the cone axis. The 2 cones of fig. 2 were from the same plant, the right hand one just before, and the other just after elongation. A cone measuring 32 cm. was found to have lengthened to 48 cm. in 3 days. Just

prior to this period of rapid elongation there is a loss of turgescence at the axial end of the sporophyll so that as the cone opens the sporophylls become loosely hinged at the centre. Dehiscence of the sporangia begins at the top of the cone and works downwards, pollen being completely shed usually in 3 or 4 days but sometimes spread over a longer period. On each sporophyll the sporangia nearest the axis open first—the reverse of the condition in Ceratozamia (2). The sporangia open back gradually so that the pollen is not shot out but simply falls on to the sporophyll below and drops to the ground, or is blown away.

Pollination.

At the time of pollination the female cones may measure as much as 15″ x 6″ with massive sporophylls 5 cm. across and nearly 2½ cm. thick. The sporophylls are closely packed before and after pollination but open slightly for a short period, the gaps between sporophylls averaging to 1 or 2 mm. Owing to the shape of the sporophyll there is, however, more space within the cone and a gap of sometimes 3-4 mm. between the axis and the tip of the ovule.

Pollination in Cycads is generally held to be anemophilous but there have been suggestions of insect pollination (Pearson, 1906). There seems no reason to doubt that pollen is transferred from the male to the female cone by wind, but from the outside of the cone to the micropyle is a distance of several cms., a condition very different from that in the conifers with their exposed ovules and wide open micropyles. Insects are found in the cones and play, I feel convinced, an important part in transferring the pollen from the cracks between the sporophylls to the micropyle. The tip of the micropyle projects up in a point so that an insect crawling about would tend to rub against it. A mealy bug (*Dactylopius macrozamii*) is found inside both male and female cones at all stages of their development, and a weevil and a small brown beetle are present in large numbers at the time of pollination.

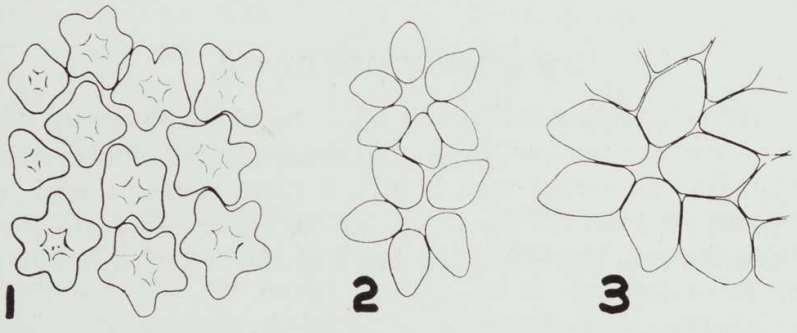
It was hoped to get some experimental evidence on the subject by sprinkling pollen on two sets of cones in one of which insects had been killed or driven out by an insecticide and comparing the resultant pollination, but the attempt had to be abandoned on account of the extreme difficulty of securing enough cones which were opened but definitely not pollinated.

I have not succeeded in finding pollination drops though doubtless they do occur. Insect pollination would not necessarily be disproved by the presence of pollination drops whose chief function is surely to draw the pollen grains down the exceedingly long micropyle.

Pollen is shed from September-October on the Coastal Plain and about a month later in the hills, and fertilization occurs four months later, by which time the cones have attained full size. Seeds are liberated February-March by the disintegration of the cone, rotting occurring at the axial end of the stalk of the sporophyll. The seeds become loosened about the same time and are usually detached from the sporophyll as it falls to the ground. If the plant is one which has developed a trunk seeds may roll for several feet but usually fall within a few inches of the parent plant and are frequently found germinating in the centre of the crown of leaves. Normally seeds lie on the ground through the whole of the next year and do not grow until after the first winter rains of the following year.

The youngest male sporophylls found were from portions of two cones dug up on March 16. The cones were 2 cm. in diameter, most of this being axis. The sporangial area could just be distinguished, measuring 1-1½ mm., but sporangia were not yet developed. Slight elevations on the surface marked the position of the centre of the sorus, and in sections these showed as heavily staining areas of meristematic cells which extended down in a wedge to link up with the vascular strands in the sporophyll. Fig. 6 shows part of a sporophyll in T.S. The same structure was described for Stangeria and Zamia by Lang (8) and Smith (12). No archesporial cells had differentiated. Subsequent history showed these cones to be far more than two weeks earlier in development than the next cones found March 31, in which the groups of sporangia were distinct. The synangial character of the sorus was very evident here, individual sporangia being little more than lobes from a central cushion—the elevation seen in the younger cones. Figs. 7 and 8 are sections through young sporangia, that in fig. 8 being slightly older. A small group of archesporial cells was present but these differed very little from the external tissues of the sporangium.

Sporangia are at the same stage of development on all parts of the sporophyll and throughout the cone. Macrozamia differs in this respect from Stangeria and Zamia but agrees with Ceratozamia.



Outline drawings of surface views of groups of sporangia—sporophylls taken from the same cone March 31st, April 14th and April 28th respectively. × 17.

Text figs. 1-3 are outline drawings of rosettes of sporangia in surface view at fortnightly intervals from March 31 to April 28, and indicate the changes in size and shape during this period. The sporangia enlarge rapidly, particularly upward and back towards the centre of the group, they become very crowded and the sides and top flattened by pressure so that the outline is almost square in T.S. The structure in May is shown in fig. 9, pl. ii., epidermis, wall of 6-7 layers of cells, the innermost already showing a certain amount of flattening, and uniform central archesporium with dividing nuclei.

A tapetum cannot be distinguished with certainty until shortly before the mother cell stage. Fig. 10, pl. ii., shows a small portion of wall, archesporium and ill-defined tapetum. There is no doubt that the tapetum is entirely sporogenous in origin. In the section of the sporongium at the stage figured some of the tapetal cells are binucleate; at a later stage this is the general condition.

A T.S. of the sporangium at the spore mother cell stage shows the epidermal cells already elongated at right angles to the surface but not yet lignified. Fig. 12, pl. ii., is taken through a L.S. of the apex of the sporangium

There may be as much as three months difference in the stage of development in plants growing in the same small area. It is obvious that with variations of this order it is only when collections are made from the same plant or from cones known to be at the same stage of development, that successive collections give the time intervals between different stages. In the case of male cones with numerous easily detached sporophylls and several cones per plant it is possible to collect over a whole season from the same plant, but female sporophylls are fewer and much larger and are difficult to remove without considerable damage to the cone. The interval between pollination and fertilisation was determined reliably this year in the case of a female plant found to be pollinated at a date when only one male plant in the locality had shed its pollen and therefore must have been pollinated from this plant. The pollen was shed on August 28th and sperms were liberated between December 28th and January 5th.

Frequency of Cone Production.

Observations made over the past few years on plants in the neighbourhood of Crawley, supplemented by more casual ones in other localities suggest that there is no regularity in cone production, or even in the growth of successive crowns of leaves. Careful records have unfortunately not been kept for long enough to give many instances of successive cones on marked plants. One female plant coned in 1930 and again in 1936, and one male in 1934 and 1938. Of about 25 plants which produced cones in 1934 or 1935 and have been kept under observation since, one male plant produced two cones again in 1938, and one male and one female are coning in 1939. In the whole area there have been cones on only five plants since 1935, and there appear to be eight or ten plants about to cone in 1939. Seedlings round two of these plants are certainly not less than 10 years old.

It seems then that for plants growing under natural conditions the interval between successive cones is usually not less than three to six years and may be considerably longer. It is probably longer in the case of female plants than male. Successive crops of seedlings round plants also furnish evidence that the average interval may be of the order of six years or more. In gardens receiving water through the summer plants may produce large and healthy female cones every alternate year, and two instances are on record of male plants coning in consecutive years.

MICROSPORANGIA.

Sporangia are more or less oval or pear-shaped with the narrow end pointing away from the centre of the sorus. When mature the wall is very hard and brittle, the epidermal cells being so heavily lignified that the cell cavity is almost obliterated. In surface view the epidermal cells are elongated in the direction of the long axis of the sporangium and have oblique end walls. In transverse section these cells are tall and narrow, becoming shorter towards the line of dehiscence. When the pollen is ripe the other layers of the wall are dry and flattened back against the epidermis. Near the apex, the epidermal cells are particularly large surrounding a slight depression occupied by a group of small lignified cells. It is this group at the end of the line of dehiscence which has been compared with the annulus of Angiopteris. Below this, and extending somewhat under the adjacent epidermal cells, is a mass of pitted cells which are heavily lignified and in striking contrast to the thin-walled sub-epidermal cells of the rest of the sporangium.

showing the lignified pitted cells and the tall epidermal cells surrounding the group of small cells in the depression. Fig. 13, pl. ii., is a photograph of a T.S. passing through the lignified cells. Nuclei are still present in these cells, whose walls become very much thicker before the sporangium is ripe. The group also becomes more extensive by thickening of adjacent cells. This figure also shows a row of cells with granular contents which occurs below the line of dehiscence.

After division of the pollen mother cells the walls of the epidermis harden rapidly and it becomes impossible to cut the sporangium by paraffin embedding methods. When the pollen grains are first formed, the tapetum is more distinct than at any other time, forming a layer of granular cells with heavily staining nuclei.

There is some disintegration of sporogenous tissue just previous to the division of the pollen mother cells. Sections show cells with small structureless nuclei among the normal archesporial cells, and in smears also these can be distinguished from mother cells by the clear nuclei and the usually less rounded shape. When division begins the microspore mother cells occupy a relatively small part of the spore cavity, the remainder being filled with liquid which stains with safranin and is coagulated by aniline blue.

MICROSPOROGENESIS.

The microspore mother cells are packed with starch grains—a condition which tends to obscure nuclear detail. Division is not simultaneous and even in one sporangium all stages may be found from resting nuclei to complete tetrads. Sporangia towards the centre of the sporophyll are slightly more advanced than those at the edges and those at the top of the cone than those at the base.

Figs. 14-18, pl. ii., are reproduced from photographs of permanent aceto-carmine smears. The long series of prophase stages can be followed well in these smears; a few are shown in figs. 14, 15 and 16. Fig. 17 and one cell in fig. 16, pl. ii., show the short thick chromosomes of the reduction division. Their tetrad nature is very apparent in some of the nuclei. Apparently some of the chromosomes are always closely associated, as chromosomes at this stage are invariably arranged in 8 or 9 groups, never 12. At the mitotic division the chromosomes are longer and more regular. Fig. 18, pl. ii., shows a cell at the anaphase of the mitotic division—the chromosomes are well separated in this preparation and 24 can be counted in each cell. The ring dividing the cell is showing in the photograph.

The peculiar type of wall formation in the pollen tetrads of cycads has been described by Juranyi, Treub and Smith for Ceratozamia and Zamia. Of these stages Smith (12) gave no figures and contents herself with the statement that most stages resembled those figured by Treub and Juranyi. Particular attention has been directed to the pollen tetrads in Macrozamia, many of the details having been observed in smears of living cells. About the time the daughter nuclei are reorganising after the reduction a thin ring appears on the wall of the mother cell in the plane of the cell plate. Two mother cells fixed and stained to show the ring in its early stages are shown in fig. 19, pl. ii. The ring gradually thickens and ultimately forms a partition across

the cell by its continued ingrowth to the centre, but the wall is not complete before the anaphase of the second division. The mother cell becomes distinctly 2-lobed as shown in the photographs.

Following the second nuclear division, walls form at right angles to the first and in a similar manner, *i.e.*, by formation and ingrowth of a peripheral ring, but this wall never approaches the first in thickness. There is, however, usually a projecting plug at one or both poles of the tetrad giving it a marked polarity (fig. 22, pl. ii.). Thickening of the first partition continues until the tetrad reaches the condition seen in figs. 20-22, pl. ii. The second walls may form in the same plane or may arise at right angles to one another, tetrads of the two types occurring in about equal numbers. In tetrads at the stages shown the exine of the pollen grain is already well developed and stains with safranin in contrast to the partition framework which takes a dense stain with gentian violet or aniline blue.

The microspore mother cell wall as a whole does not thicken at all and at the 4-celled stage can frequently be seen in tetrads mounted in water as in figs. 21 and 22, pl. ii.

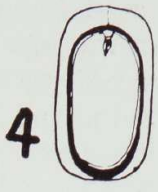
The pollen grains round up as their membranes thicken and the tetrads take the form shown in figs. 20-22, pl. ii. At this stage spores are easily detached from framework and in smears mounted in water many can be found just breaking away from tetrad as in fig. 23, pl. ii. Wall structures can be seen very well in the empty framework (fig. 24, pl. ii.). The heavy first wall is still relatively thin at the centre—very thick at the circumference. The vertical walls are similarly thin in centre with a heavier rim particularly thick where it joins the edge of the horizontal wall.

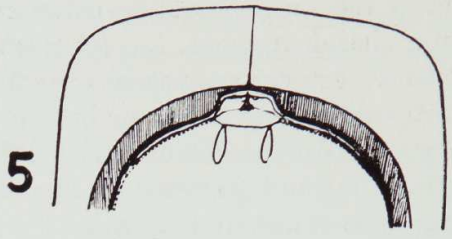
Slightly later, when the pollen grains are free, no sign can be found of this framework, and it was not until the second year of examination of tetrads that some light was thrown on the problem of its disappearance. If smears are made with sufficient care from a sporangium containing both tetrads and free spores, some groups can be found still enclosed in the mother cell wall, but with the structure of the cross walls completely destroyed. These are evidently some pectic compound which gelatinises when the tetrads are mature. Fully developed tetrads heated in water swell enormously, and the pollen grains become free, or hang together in groups of four surrounded by a structureless gelatinous mass. If the warming is very gentle it is possible to get the tetrads in a condition in which it can be seen that it is in the cross walls only that swelling has occurred. These are elongated and buckled as the whole structure is still enclosed in the unchanged mother cell wall.

The pollen grains are uninucleate when the tetrads break up, and it is not until a month later that the prothallial cell is cut off. The division of the antheridial cell follows after another 3-4 weeks and two weeks before the pollen is shed, making a total of 10 weeks or more between the formation of the pollen grains and their liberation. Some dividing nuclei were seen at each cell division but are difficult to distinguish clearly through the wall of the pollen grain. There is still liquid in the sporangium at the time of the first division in the pollen grain but the whole sporangium dries gradually before it opens. When shed the pollen grains are dry and folded in on one side, that remote from the prothallial cell, so that the grains appear elliptical. They absorb water readily however, and will rapidly swell out to the spherical form.

OVULE.

The ovule is bright red when mature, measuring 4½ cm. x 5½ x 3½ — 4, with the sides flattened where in contact with adjacent ovules.





Text fig. 4.—L.S. Seed at time of shedding. × 1/3.
Text. fig. 5.—Upper part of ovule Dec., stony layer of integument shaded.

The seed conforms to the standard cycad type as shown by text figures 4 and 5, which give the relative proportions of the various layers. The outer fleshy integument is pigmented throughout, and is traversed by an elaborate series of anastomosing mucilage canals easily visible to the naked eye. The vascular system consists of unbranched or rarely branched strands in the outer integument and more numerous branching and anastomosing strands in the inner. The stony layer is about 1½mms. thick at the sides of the ovule and double that thickness at the base, where it is penetrated by the few main strands to the inner integument. At the apex of the seed there is a thin area surrounding the micropyle bordered by a particularly heavy rim, so forming a hollow into which the thick central region of the nucellus fits. The structure of this region of the ovule is shown in text fig. 5. This is marked by radiating lines which are conspicuous from a very early stage in the development of the ovule. The nucellus is free from the integument only in the upper part of the ovule and when the seed is mature is reduced, except in the centre, to a thin papery membrane.

Archegonia number from 3 to 9, usually 4-7, arranged in a circle round the edge of the depression at the apex of the prothallus. Several instances, however, of abnormal distribution of archegonia have been seen. In some they are in a double or triple circle at the apex, in others in an irregular group, and in one cone a number of the ovules had a very much elongated lateral group with 12-15 archegonia. Treub records a double archegonial group in Cycas circinalis as an exception. These cases of irregular and more numerous archegonia are of interest in view of the fact that in Microcycas large numbers of scattered archegonia occur.

Development of the Ovule.

The youngest ovules examined were from fragments of 2 cones collected early in March. The cones at this time are very deeply buried and only 6 undamaged ovules were obtained. These were 2 mm. in diameter by a little over 1 mm. long. The integument had already closed over the nucellus and in all except one ovule the megaspore was at an early free nuclear stage. Figs. 25 and 26, pts. II. and III., are median sections of two of these ovules.

Surrounding the megaspore there is an extensive zone of spongy tissue, which is sharply delimited from the remainder of the nucellus, and in which the cells have the appearance of sporogenous tissue with heavily staining cytoplasm and large nuclei. The spongy tissue grows with the rest of the ovule as the megaspore enlarges, but is gradually used up

and disappears by the time of cell formation in the prothallus. At an intermediate stage the outer layers are of compact cells with normal nuclei while near the embryosac the cells are degenerating. The megaspore membrane tends to pull away from this loose tissue, making fixing difficult.

At a late free nuclear stage an irregular layer, usually two cells deep, can be distinguished at the edge of the rapidly disappearing spongy tissue. These cells are granular and occasionally binucleate and constitute the tapetum. By the time cell formation in the prothallus is completed this layer consists of empty cells with suberised walls, in which condition it persists throughout the development of the prothallus.

Figs. 26 to 29, pt. III., indicate the changes taking place in the ovule between March and September. The layers of the integument gradually differentiate as the ovule enlarges, mucilage canals and tannin cells appear, the vascular strands become distinguishable and a very thick cuticle develops both on the epidermis of the ovule and on the surface of the free part of the nucellus.

The pollen chamber does not appear until shortly after cell formation in the prothallus, although that region stains heavily for some time before. A couple of weeks before pollination the passage through the nucellar beak is complete as shown in fig 29, pt. III., the remains of the beak fitting closely in the lower half of the long narrow micropyle.

From September onward the ovule enlarges rapidly, pigment develops in the outer integument and mucilage canals become very conspicuous. The stony layer hardens slowly and can be cut with a knife until early December. The whole cone is very compact and the sides of the ovules flattened by pressure. By January or February the ovule has reached its maximum size, the integument is fully differentiated and the nucellus and inner integument papery.

Ovules which have not been pollinated do not continue their development for long and the prothallus does not become starchy, but except in the case of isolated plants, failure of pollination is surprisingly rare.

Another source of abortion of the ovules is failure of the megaspore to develop. In some cones there is a high percentage of ovules which never grow beyond 2-3 cms. Until the stage of fig. 27, pt. III., these ovules develop normally except in the central region, which is cellular throughout instead of being occupied by the large free nuclear megaspore. In the absence of the megaspore the spongy tissue keeps pace with the growth of the ovule for a time, later cracks develop, and the ovule tends to collapse in at the sides

FEMALE GAMETOPHYTE.

The megaspore mother cell has not been seen. The nucellus of the smallest ovule found is shown in fig. 25, pt. II. There is in this a very narrow elongated uninucleate megaspore evidently the lowest of a vertical row, the remains of the other three showing at the upper corner. The cytoplasm is very scanty and the single nucleus small. It is possible that this was an ovule in which the megaspore was not going to develop further. In the other ovules the megaspore was at an early free nuclear stage, but fixing was not good enough for details of the nuclei to be seen. Fig. 26, pt. III., a median section of one of these ovules, shows the nucellus with the zone of spongy tissue, compact in its outer layers but made up of loose cells towards the centre where it touches the megaspore membrane.

The free nuclear period lasts from March-April to July or August. Throughout this period the increase in the amount of cytoplasm just keeps pace with the enlargement of the cell and is never more than a thin film scarcely the depth of a nucleus. The nuclei are evenly distributed through the cytoplasm and just previous to wall formation are connected by conspicuous radiating lines in the cytoplasm which forms a thicker layer at this stage. Fig. 30, pt. III., shows part of the edge of the megaspore from another section of the ovule of fig. 28, pt. III. The cytoplasm with its contained nuclei is in its natural position against the megaspore membrane and the remains of the spongy tissue lie between the membrane and the bi-nucleate cells of the tapetum.

Cell formation is initiated when the embryosac is about 7 mm. x 4-5 mm. and there are in the neighbourhood of 1,000 nuclei in the embryosac. Cell formation in the prothallus was described by Light in 1924. I was not fortunate enough to see the simultaneous mitoses, but can confirm her account of other steps in the process.

The nuclei are partitioned off by walls which form on the cell plates of the last free nuclear division, but are open to the central vacuole. At the next division walls are formed to give a layer of small cells against the membrane and a layer of open cells whose cytoplasm is in contact with the liquid of the central vacuole. When an embryosac is dissected out whole from a fresh ovule and examined in optical section this peripheral layer of cells can be readily distinguished. The living cells, cytoplasm and nuclei can be examined when such a prothallus is cut and spread out on a slide. The next layer of cells is formed in a manner similar to the first, and growth proceeds towards the centre, always with free nuclei at the inside edge of the advancing cells. With the exception of the first layer, these cells are long with very scanty cytoplasm, and apart from an occasional cross wall are only 3-4 layers deep when they meet at the centre. Cross walls form in these long primary prothallial cells from the outside inwards. One ovule examined had numerous divisions in the outer third, a few thin walls in the next third and undivided cells just meeting in the centre. The line where cells from opposite sides meet is very evident at this stage, but soon disappears. Growth of the prothallus is relatively slow so that it is not difficult to find incompletely cellular stages. Up to pollination the prothallus is very soft, but after that event becomes firmer, and within a month or six weeks starch begins to appear in the cells, first showing as a few small grains round the nucleus, then in the peripheral cytoplasm, and gradually increasing until by fertilization the cells are closely packed with large grains.

The megaspore membrane is thin in early free nuclear stages, up to cell formation the outer layer is thin in comparison with the inner. The membrane thickens rapidly during the growth of the prothallus and the outer layer, made up as in other cycads of a close pile of clubbed rods, greatly exceeds the inner one in thickness.

Archegonia.

The development of the archegonium has been fully described for other Cycads and appears to be quite typical in *M. Reidlei*. Archegonial initials appear shortly after wall formation in the prothallus and a primary neck cell is cut off from the central cell. The young archegonium has a central vacuole with a thin peripheral film of cytoplasm. The central cell nucleus

lies immediately below the pair of thin, flat neck cells. The cells surrounding the archegonium are differentiated as a jacket layer which becomes more distinct as development of the archegonium proceeds.

By the time of pollination, the archegonia are just large enough to be seen with the naked eye, but there is still a single central vacuole. In the period between pollination and fertilization the cytoplasm increases in amount and changes from a peripheral layer to a highly vacuolated mass throughout the cell, the vacuoles towards the centre being very much larger than at the edge. As the archegonium approaches maturity the cytoplasm becomes much denser and the vacuoles practically disappear. Such changes during the growth of the archegonium have been figured by Chamberlain for Ceratozamia (7) and Lawson for Bowenia (9).

The neck cells are conspicuous throughout development, showing in surface view as a pair of large semi-circular discs many times the diameter of the surrounding cells. They are astonishingly thin in comparison with their width, their depth rarely exceeding that of the contained nucleus. Shortly before fertilization the neck cells become inflated and project into the archegonial chamber as a pair of balloon-like structures easily seen under a very low magnification.

The central cell nucleus remains in position below the neck cells, but enlarges considerably. Its division was not seen, but one preparation showed what might have been the remains of the ventral canal nucleus.

The egg nucleus lies in the centre of the archegonium, and as in other Cycads is extraordinarily large, measuring 500 x 300 μ. No figures are given of the unfertilized archegonium, but its general organisation can be gathered from figs. 36 to 38, pt. III., of the proembryo. The egg membrane is thick and pitted as in other genera. Fig. 35, pt. III., shows a small part of the membrane in section with the egg cytoplasm filling the pores.

The apex of the prothallus is at first above the ring of archegonia, but flattens as the prothallus enlarges and the ring expands, and then during the month preceeding fertilization the archegonial chamber forms as a depression, reaching a depth of 1 mm. Occasionally each archegonium lies in a secondary depression.

MALE GAMETOPHYTE.

Sections of the nucellus a few days after pollination show pollen tubes beginning to penetrate the tissue surrounding the pollen chamber. The exine ruptures on the side away from the prothallial cell and a short tube with its nucleus grows out, the prothallial and generative cells remaining as in the ungerminated grain. As many as 20 (occasionally more) pollen grains are found in each nucellus. In a cone which was smothered with pollen on September 26th and sectioned September 30th over 100 germinating grains were counted in one pollen chamber. The photograph fig. 31, pt. III., was made from a thick hand section of this nucellus. The two tubes in focus show the nuclei of the generative and prothallial cells.

Shortly after this the generative cell divides into stalk and body cells, the latter small, and without the very dense cytoplasm characteristic of later stages. During the next few weeks the tubes push deeper into the nucellus, keeping parallel with its upper surface. The extent of the growth of the tubes can be followed by the lines of darkened tissue round each tube, which show on the upper surface of the nucellus. A pollen tube from a nucellus fixed November 13th, is shown in fig. 32, pt. III. The body cell

is now clearly defined with a large nucleus and dense cytoplasm. The blepharoplasts (only one showing in the section) are still small and are lying in the direction of the long axis of the tube. The prothallial cell has pushed up into the stalk cell the nuclei of the two now lying side by side, the exine of the pollen grain still adhering to the end of the tube. The tubes are packed with starch and are unbranched or have one or two short branches, little more than lobes, near the far end. The tube nucleus during the growth of the haustorial part of the tube lies near the tip, but before the sperms are formed moves back and is found at the prothallial end of the tube.

For the first three months there is little change in the pollen chamber but during the month preceeding fertilization the prothallial end of the tube enlarges and grows down through the rapidly disintegrating tissue in the centre of the nucellus. The body cell nucleus enlarges enormously and the cell becomes spherical, the blepharoplasts change from small specks to large vacuolate structures and move round through 90° to lie across the axis of the tube. No attempt was made to get a close series here. The development of body cell and blepharoplasts and the formation of sperms has been fully described by Chamberlain and Lawson for other Cycads, and the stages seen indicate that Macrozamia is in no way peculiar. The actual division of the body cell was not seen, but sections of the pair of sperms in the tube show that these are formed inside the mother cell.

Sperms.

The living sperms were first observed in January, 1930, by Miss A. Fabre and have been examined in subsequent years by the author. The sperms are of the usual type with a ciliated band round the upper part and an extraordinarily large nucleus surrounded by a narrow sheath of cytoplasm. The ciliated band is deepest at the tip diminishing towards the lower coils. The spiral band is highly refractive and stains heavily in sections. The cilia originate on the band and pass through a small amount of cytoplasm between the band and the surface of the sperm. Sperms of *M. Reidlei* measure 210-220 μ. in diameter, *i.e.*, larger than those of Cycas but smaller than in Zamia. Fig. 33, pt. III., is taken from a tube mounted whole in Venetian turpentine and shows the stalk and prothallial cells with the pair of sperms still enclosed in the mother cell. In one living pair watched at this stage, there was distinct vibration along the band but no cilia visible. As a few minutes later the cilia could be seen, it was concluded that the mother cell wall, intact at first, had been ruptured by the vibrating cilia. The mother cell wall has invariably disappeared before the sperms begin to rotate. Several pairs of sperms were watched at this stage. There was no rotation of the sperm as a whole, but the sensitive tip was slowly moving in a circle. Miss Fabre watched a pair of sperms in motion in the tube for two hours—beyond this we have no information as to the duration of the period of motility. The sperms eventually break free from each other and swim up and down the tube, rotating rapidly with the apex directed forward. They are spherical except for the beak, but are soft and readily change shape when they come in contact with the sides of the tube or jam in the narrow upper part. Fig. 34, pt. III., shows three tubes fixed after the sperms had started to move. In the tube on the left the pair has just become detached from the stalk cell, in the central one the two sperms are side by side at the base of the tube, and in the right-hand one the sperms have moved up out of sight under the nucellus.

FERTILIZATION AND EMBRYO.

The fertilized egg can be identified with certainty by the presence of the ciliated band of the sperm, usually near the top of the egg, but occasionally in the lower half, as has been found also in Dioon and Zamia. One slide only has been obtained in which the two nuclei are in contact, the smaller sperm pressing in on the upper side of the large egg nucleus. A number of slides show a single very large uniformly staining fusion nucleus. The actual division of this was not observed. The cytoplasm of the fertilised egg is smooth and dense, except for a disturbed area which marks the passage of the sperm nucleus.

The entry of the sperm into the egg has been described by Chamberlain for Dioon and by Lawson for Bowenia. As in those genera, the neck cells in Macrozamia are still intact after the entry of the sperm as shown in fig. 36, pt. III. In some fertilized eggs there is, immediately below the neck cells a heavily staining mass which, in fresh ovules, shows as an oily substance. This was noticed by Treub in Cycas. It seems likely that this is the remains of a second non-functional sperm. The one in fig. 36, the only recognisable second sperm seen in an egg, is already showing the red staining characteristic of disorganising tissue. No case of several sperms inside one egg, as recorded for Stangeria and Bowenia, has been found.

Early divisions apparently take place in rapid succession as of numerous eggs sectioned practically all had the single fusion nucleus or a large number of free nuclei. Only one two-nucleate proembryo has been seen. In ovules containing multinucleate proembryos, some of the eggs, presumably those which have missed fertilization, have a large central vacuole and smaller ones in the lower part. At later stages these archegonia are left as empty shells, the egg membrane still retaining its shape.

Free nuclear stages of the proembryo show the nuclei evenly dispersed through the smooth cytoplasm. Several preparations were obtained showing simultaneous mitoses in the proembryo. The L.P. photograph, fig. 38, pt. III., was taken from one of these sections. As in other cycads, the division figures are remarkably small in comparison with the resting nuclei.

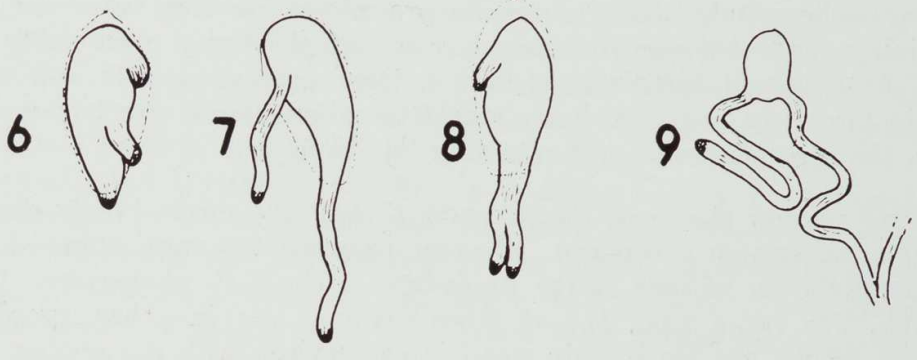
Fig. 37, pt. III., shows nuclei at the apex of the proembryo after one of the late divisions. The neck cells are in the collapsed condition found soon after fertilization. The cytoplasm is becoming visibly vacuolate again. No sign of evanescent walls has been seen before the true walls form (*cf.* Dioon.)

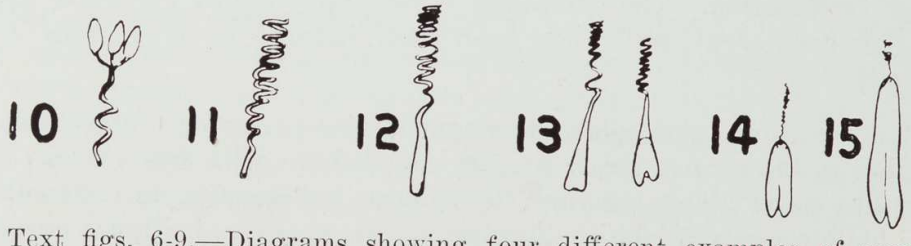
Wall formation takes place throughout the proembryo except for a small region in the centre, which remains non-cellular with free nuclei. This region breaks down about the time the embryo is beginning to differentiate, leaving a hollow cavity, which is shown in fig. 41, pt. III. In *Cycas circinalis* (14) *C. revoluta* (Ikeno) the breakdown of the central tissue occurs much earlier, even before cell formation, so that the nuclei are restricted to a peripheral layer of cytoplasm and the cellular tissue when it does form is never as extensive as in *M. Reidlei.* In *M. Moorei* the proembryo is completely cellular, also in Encephalartos. Probably a series could be traced through species of Cycas, Macrozamia and Encephalartos, showing progressively earlier wall formation in relation to the appearance of the central cavity.

There are further divisions in all parts of the cellular proembryo, but these are not simultaneous. In one set of preparations most nuclei were dividing in the upper part while those in the lower were resting. These gave a good series of mitotic figures, one of which is shown in fig. 39, pt. III. The chromosomes are straight and slender.

The embryo proper develops as a result of active division of a few cells at the base of the proembryo and the suspensor from the cells immediately above these. Fig. 41, pt. III., shows the suspensor cells just distinguishable above the small mass of embryo cells. Elongation of the suspensor pushes the rest of the proembryo back into the egg and the embryonic tip down into the prothallus. In fig. 42, pt. III., the suspensor has begun to elongate and the embryo has just broken through the egg membrane (showing as a dark line round the proembryo). The central cavity has now reached its maximum size. The archegonial membrane retains its shape and can be dissected out and handled with ease until the time when it is crushed back by the enlarging embryo.

In *Macrozamia Reidlei* from 1-3 lateral embryos have been found in addition to the normal basal embryo. These develop in exactly the same way as the basal one, *i.e.*, from a small group of actively dividing cells in one particular region of the periphery of the proembryo. Development in these apparently does not proceed very far, as they have only been observed at early stages. When lateral embryos have just begun to elongate they are still inside the egg membrane and are likely to be missed if this is not





Text figs. 6-9.—Diagrams showing four different examples of several embryos developing from one zygote. (Dotted line indicates the position of the egg membrane.)

Text figs. 10-15.—Diagrams showing development of the embryo; fig. 10—March, 11—May, 12—July, 13—September-October, 14—December, 15—February. (14 and 15 half previous scale.)

removed before examination of the embryo. Four different arrangements of multiple embryos are shown in Text figs. 6-9. In Text fig. 8 there is one small lateral and two equally developed basal embryos, which in this

case appear to represent simply a forking of a single suspensor. Branching of the suspensor has been recorded by Saxton for Encephalartos, and it would be interesting to know if lateral embryos also develop in this genus. It is only in those genera in which the proembryo consists of uniformly cellular tissue that polyembryony of exactly this type could arise. The condition is not rare in *M. Reidlei* as it was found in about half the ovules examined from two cones, occurred in two others of those examined in 1937, and was seen in seeds from two cones dissected this year.

Embryos from several archegonia meet and their suspensors become closely associated. One embryo, however, grows more rapidly than the others, which are left behind at various levels up the suspensor. As growth proceeds, the number of cells across the embryo, and correspondingly, the width of the suspensor, increase. The upper part of the suspensor dries and its coils are pressed back against the micropyle, so that in later stages only the lower most recently formed part is turgid. It would seem that the significance of the long suspensor is the insuring of the existence of a turgid length of suspensor, which up to a point increases in thickness with the increasing size of the embryo. It must be remembered in this connection that the embryo is growing in the endosperm for over twelve months after the seed has been shed. The average length attained by the suspensor is about 9 cms. From March to June the embryo remains a small mass of cells at the end of the elongating suspensor, July to September there is a noticeable enlargement, and by October cotyledons are just forming in most seeds. Not long after the appearance of cotyledons meristematic activity begins at the suspensor end to form the coleorhiza and root apex. The cotyledons close over the stem apex and grow steadily down through the endosperm, ultimately occupying two-thirds of the length of the seed. Text figs. 10-15 show the growth of suspensor and embryo at intervals from March to February. During this period development lags in a number of seeds, most of which will never be capable of germinating.

The number of cotyledons is normally two, but two embryos with three have been seen, one at an early stage when the cotyledons had just appeared and the other in a mature seed.

The very interesting results of klinostat experiments by Sister Helen Angela suggested that the single cotyledon in Ceratozamia might be related to the fact that seeds were shed before the cotyledons developed and were lying in a horizontal position during the development of the embryo. If that is true, the suppression of one cotyledon has not followed early shedding in Macrozamia and Cycas, both of which shed their seeds some months before cotyledons appear.

GERMINATION AND SEEDLING.

There is no dry or dormant stage in the development of the seed. By April or May most embryos have reached the maximum size attained inside the seed, and the coleorhiza is pressed back against the thin area of the testa surrounding the micropyle. The radiating lines in this area mark definite structural weakness which has been increased by a certain amount of decay during the year on the ground, so that when the root is forced against it the testa cracks open into a number of wedge-shaped pieces readily forced apart by the growing embryo.

The embryo pushes out until the cotyledons are 1 cm. beyond the end of the seed, but without any elongation of the root. This growth is quite independent of external moisture and takes place equally well in seeds

which have been kept on a shelf in the laboratory or enclosed in boxes. Unless moisture is available at this stage however, no further growth can take place—seeds which have started to germinate before the winter rains shrivel and die—but under favourable conditions the root next begins to grow and breaks through the fibrous coleorhiza.

Thus, in the present year, seeds which germinated at the end of April produced roots which grew slowly through the winter, being 5-8 cm. only in August, the first leaf appearing early in November, by which time the roots were 20-25 cms. long. As in other Cycads, young plants grow very slowly, producing but a single leaf annually for many years. The seedlings in fig. 5, pt. I., are one and two years old respectively. Since the seeds germinate on the surface, while plants four or five years old have the stem apex several inches below ground level it is evident that the tap-root must contract considerably during its growth. In the juvenile leaves the pinnae are serrated near the tips, as in many other species. The number of pinnae increases in successive older leaves but varies considerably in plants of the same age, and is quite unreliable as an indication of the age of a young plant.

The anatomy of the seedling was not investigated beyond the determination of a solid cotyledonary vascular plate as in other genera and the usual girdling leaf traces.

CONCLUSION.

The general course of the life history and the structure and development of the reproductive organs, as described above for M. Reidlei are typical of the cycads as a whole. A point of rather special interest in Macrozamia is the occurrence of a type of polyembryony not, as far as I know, previously recorded. The development, structure, and ultimate dissolution of the partition walls in the pollen tetrad have also been shown to present features of extraordinary interest.

In certain features, viz., the high spore output per sporangium and large number of nuclei in free nuclear stages; the advanced condition of the female prothallus at the time of pollination; undifferentiated proembryo, immature state of the embryo when the seed is shed, and comparatively small sperm, Macrozamia is probably correctly considered as one of the more primitive cycads.

Comparison has been made with other genera throughout the paper and it is suggested that the affinities of Macrozamia are with Cycas and Encephalartos, being probably closest to the latter, which it resembles in general habit and structure of cones and seeds. In this connection it is worthy of note that Cycas (Australia and Orient) and Encephalartos (South Africa) are its nearest neighbours geographically.

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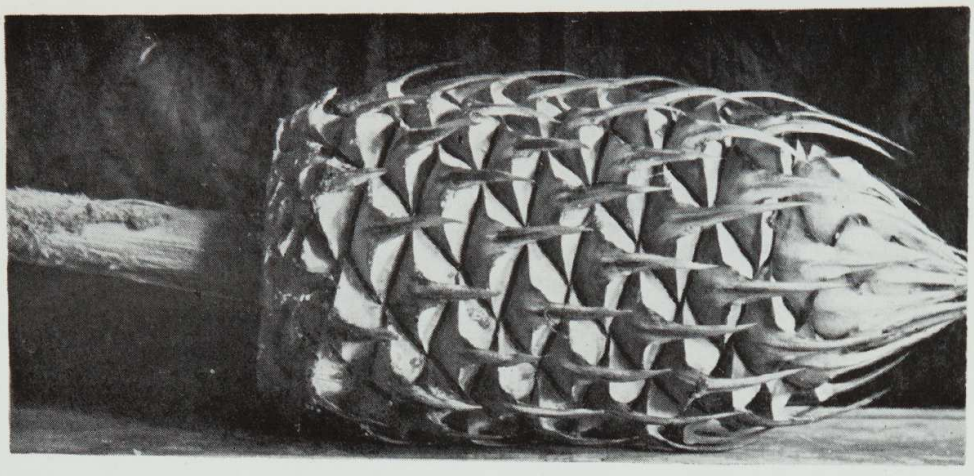
ILLUSTRATIONS.

Plate I.

Fig.

1. Mature female cone—February.
2. Male cones: that on the right just before, and on the left just after the elongation preceding the shedding of the pollen—October.
3. Male sporophylls:—the L.H.S. upper one with sporangia open after shedding pollen. The lower row from left to right shows sporophylls from top, centre, and base of a cone. On the L.H.S. sporophyll a few sporangia are open.
4. Young female cone and two detached sporophylls with minute ovules—April 10.
5. Seedlings one and two years old respectively.



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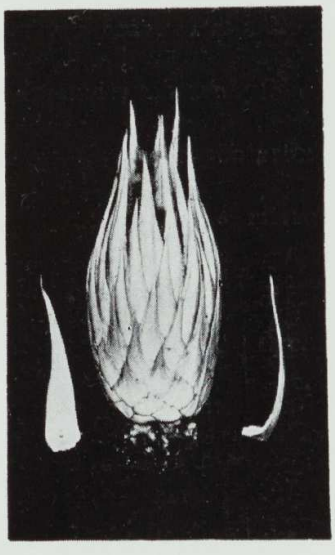

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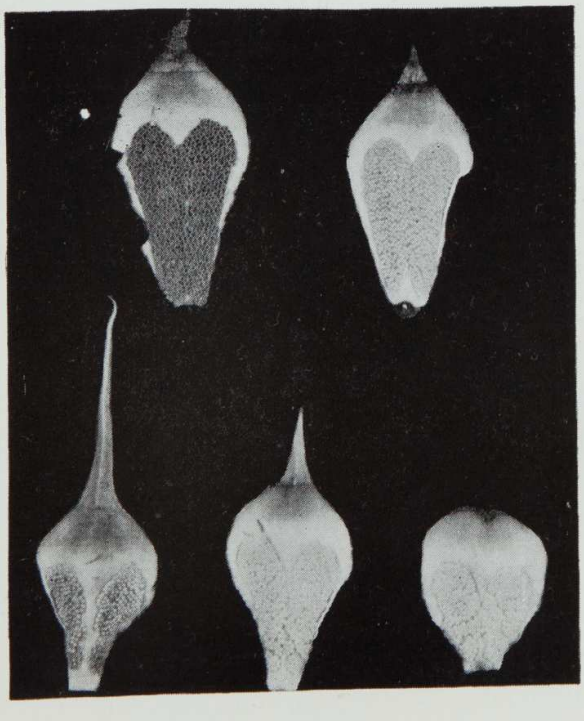

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PLATE I.

Plate II.

Fig.

6. T.S. young sporophyll showing early stages of sorus; meristematic areas running down to the vascular strand of the sporophyll. × 70.
7. Section through sporangia at the stage of text fig. 1—March 31. × 70.
8. Section through slightly older sporangia. × 70.
9. T.S. sporangium—May 11.
10. Part of wall, tapetum and archesporium—May 26. × 175.
11. Same at Megaspore mother cell stage—June 9.
12. L.S. apex of sporangium.
13. T.S. same region passing through group of lignified cells.
14. }
15. } Prophase stages of 1st division in the pollen mother cells. × 385.
16. }
17. Chromosomes of the meiotic division.
18. Chromosomes at anaphase of mitotic division in the daughter cells.
19. Pollen mother cells stained to show early stages of the ring.
20. }
21. } Pollen tetrads photographed from unfixed smears.
22. }
23. Pollen grains just becoming detached from the framework of the tetrad.
24. Empty framework of a tetrad in which the second walls are at right angles to one another, and a pollen grain in optical section showing exine and intine.
25. Median section of central part of young ovule showing part of integuments, nucellus, and spongy tissue.

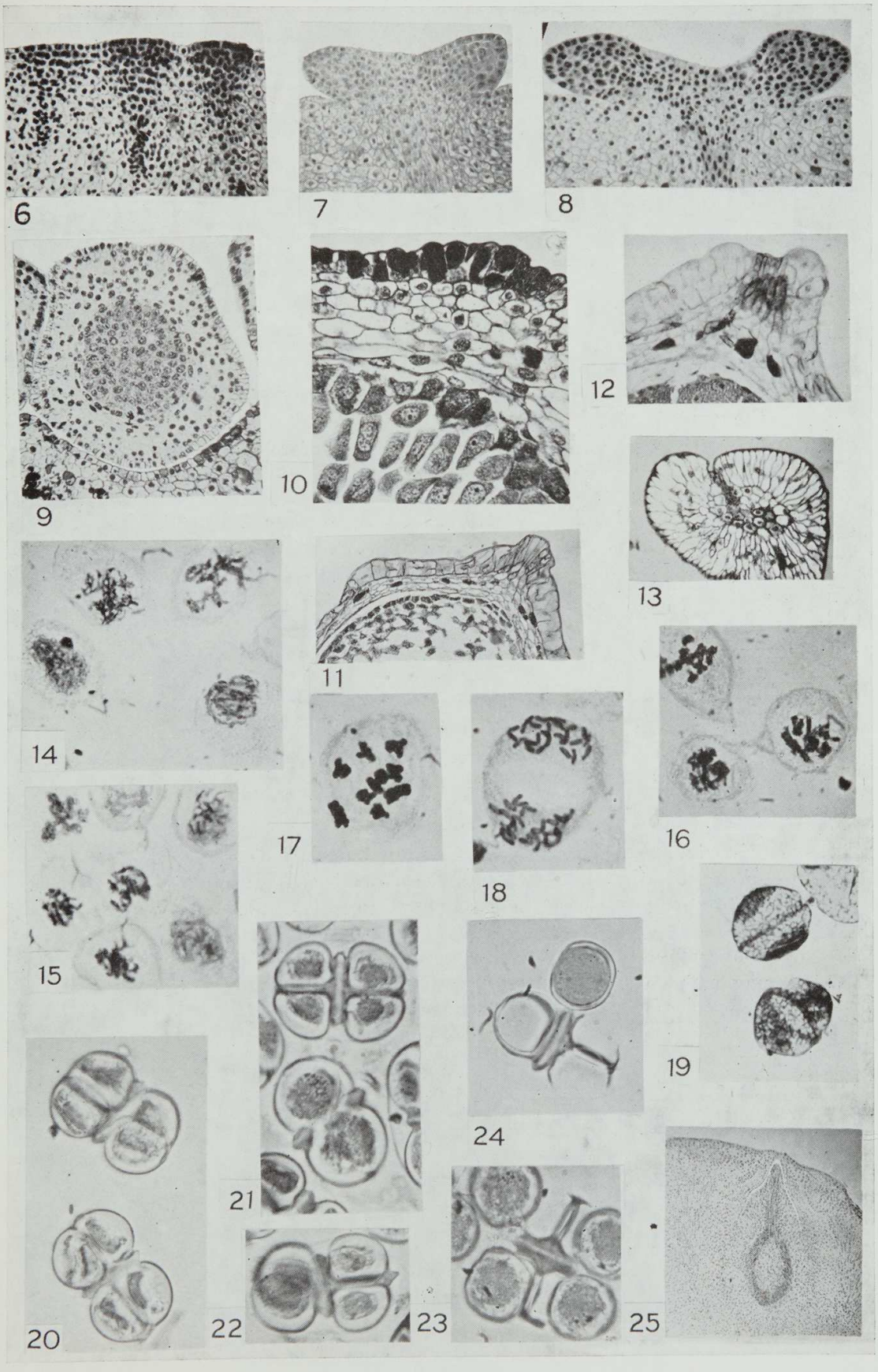

PLATE II.

Plate III.

Fig.

26. Median L.S. young ovule, early free nuclear megaspore March 16. × 6.
27. L.S. slightly older ovule, megaspore membrane shrunk into the centre of the ovule. × 8.
28. Ovule with free nuclear embryosac just before cell formation—May. × 6.
29. Upper part of ovule shortly before pollination, nucellar beak in micropyle, pollen chamber, tapetum, megaspore membrane—October. × 8.
30. H.P. of small portion of ovule of fig. 27 showing cytoplasm with free nuclei against megaspore membrane; remains of spongy tissue, tapetum and the edge of the nucellus. × 175.
31. Pollen grains germinating in pollen chamber four days after pollination.
32. L.S. nucellus with pollen tube, showing exine of pollen grain; prothallial cells; body cell with large nucleus, dense cytoplasm and blepharoplast.
33. Photograph of whole tube with pair of sperms. × 80.
34. Three tubes fixed after the sperms had started to move.
35. Small section of egg membrane showing cytoplasm of egg in the pits, jacket cells outside.
36. Apex of archegonium shortly after entry of sperm.
37. Apex of free nuclear proembryo.
38. Multinucleate proembryo with dividing nuclei.
39. Cellular proembryo showing absence of cell walls in centre. Section torn.
40. A dividing nucleus from a cellular proembryo.
41. Differentiation of embryo, suspensor cells just beginning to elongate above embryo proper, central cavity formed.
42. Young embryo and suspensor.

Figs. 36 and 37 × 70. Figs. 38, 39, 41, 42 × 11.

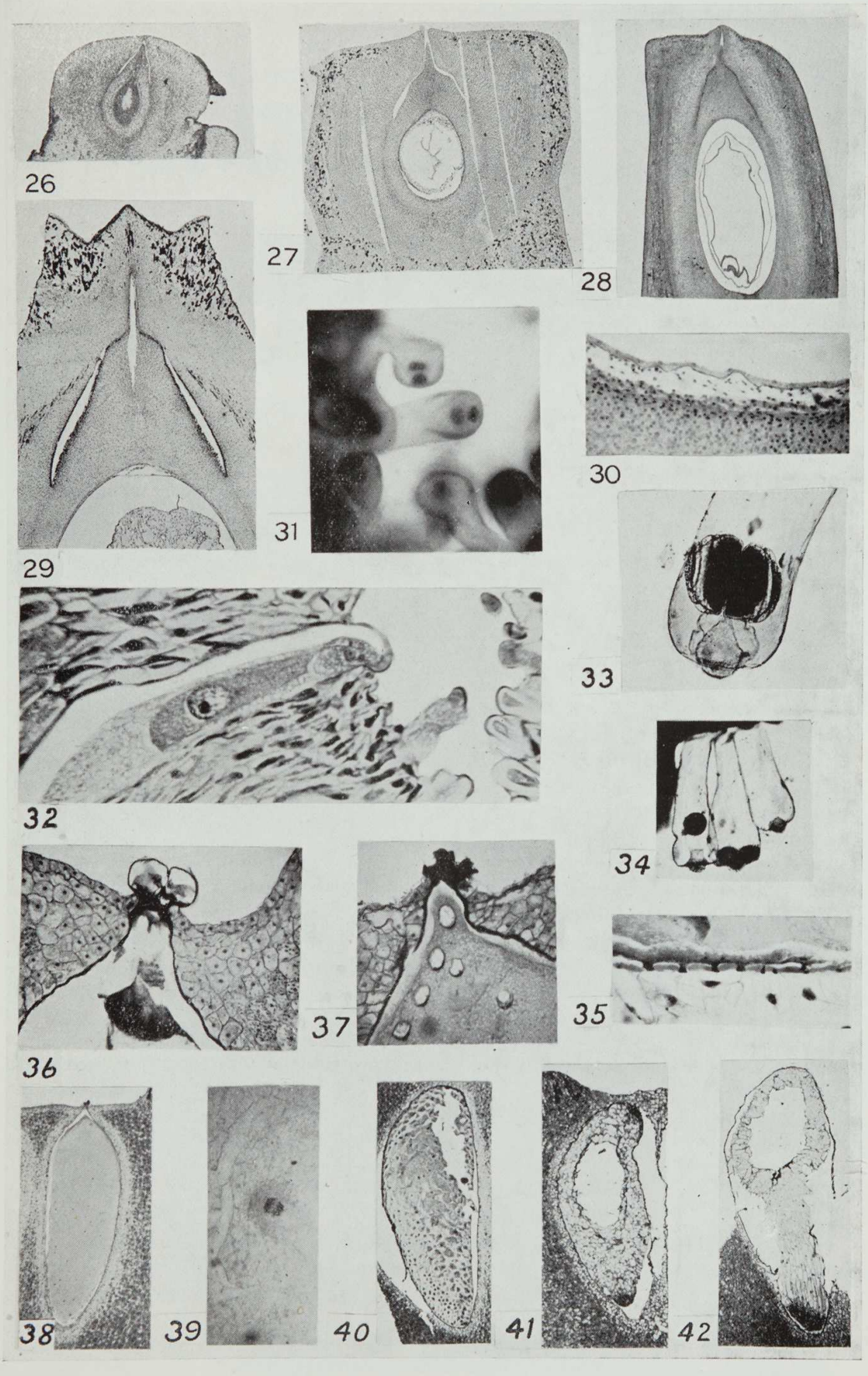

PLATE III.

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